Int. J. Morphol.,
42(5):1347-1354, 2024.

Impact of Sexual Maturity and Seasonality on Immunolocalization of S100 and Alpha Smooth Muscle Actin in the Efferent Ductules of the Dromedary Camel (*Camelus dromedarius*)

Impacto de la Madurez Sexual y la Estacionalidad en la Inmunolocalización de S100 y Actina de Músculo Liso Alfa en los Conductillos Eferentes del Camello Dromedario (*Camelus dromedarius*)

Mohamed E. Alkafafy

ALKAFAFY, M. E. Impact of sexual maturity and seasonality on immunolocalization of S100 and alpha smooth muscle actin in the efferent ductules of the dromedary camel. *Int. J. Morphol., 42(5)*:1347-1354, 2024.

SUMMARY: The study was designed to explore the association between the immunolocalization of alpha-smooth muscle actin (α-SMA) and S100 proteins within the efferent ductules (ED) from one side, and the sexual maturity and seasonality from the other side. For each season, tissue specimens were obtained from 7 adult and 7 juvenile, clinically healthy male dromedaries. Specimens were investigated by the standard immunohistochemical procedures. The immunohistochemical findings displayed that the pattern of expression was similar for both proteins, either in adults or in juveniles. Expression was significantly higher in adults during season of sexual activity (winter), fairly reduced during periods of transition from activity to inactivity (spring) or from inactivity to activity (autumn) and reaches its lowest magnitude during season of sexual inactivity (summer). On the other hand, a moderate immunoreactivity of both proteins in ED from juvenile male camels was nearly uniform throughout the year. The immunoreactivity for both α-SMA (smooth muscle cells in ductular and vascular walls) and S100 (ductular ciliated cells), was evident during periods of sexual activity. It can be concluded that the immunoreactivity is androgen-dependent and positively correlated with both of sexual activity and maturity, suggesting a crucial role of both α-SMA and S100 proteins in the regulation of the diverse functions of the ED in male dromedaries.

KEY WORDS: Dromedary camel; Efferent ductules, α–SMA; S100; Immunohistochemistry.

INTRODUCTION

Dromedaries have a great economic value in the arid regions as found in the region of the Middle East. This economic importance results from their contribution as a source of meat, milk, hides and wool, as well as their use as a means of transport (Farah, 2004). Additionally, the camel's milk may be used in traditional medicine for curing many diseases (Ibrahim *et al.*, 2016).

Many publications have studied the seasonal changes in the histology and histochemistry of the reproductive system in male camels in the last three decades of the 20th century (Tingari & Moniem, 1979; Singh & Bharadwaj, 1980; Tingari *et al*., 1984; Tingari, 1989; Zayed *et al.*, 1995) and the other continue in same line throughout the current century (Zayed *et al*., 2012; Ahmed *et al*., 2013; Ibrahim, 2015; Ibrahim *et al*., 2017; Abdel-Maksoud *et al*., 2019; Ibrahim & Abdel-Maksoud, 2019; Ibrahim *et al*., 2021; Alkafafy, 2022). Due to the retaining of their reproductive capacities throughout the year, male dromedaries are described as atypical seasonal breeders (Zayed *et al*., 1995). Accordingly, there are slight seasonal variations in the histologic and morphometric features in the male reproductive system in breeding and non-breeding seasons (Zayed *et al*., 2012).

Efferent ductules extend from the rete testis at the upper part of the mediastinum testis and converge forming a single epididymal duct. Although ED have been described as a portion of the epididymis, they have some peculiarities such as the smaller luminal diameter and the lower epithelium (Zayed *et al*., 2012). ED are lined with columnar epithelium with both ciliated and non-ciliated cells, displaying wavy-shaped cross sections profiles of ED (Ibrahim, 2015). Although it had been described as a channel conveying sperms from the testis to the epididymis, ED have a wide range of functions including absorption, secretion, transport, as well as endocrine and metabolic functions (Ilio & Hess, 1994).

Department of Biotechnology, College of Science, Taif University, Saudi Arabia.
FUNDING. This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-109).

Received: 2024-04-28 Accepted: 2024-06-06

S100 proteins are a set of strictly related, small, acidic proteins with water solubilty (Zimmer *et al*., 2013). They belong to a subfamily of Ca^{2+}-binding proteins with a wide array of biological activities (Heizmann *et al*., 2002; Cruzana *et al*., 2003).

Alpha-SMA is an actin isoform, which belongs to the contractile proteins and is a reliable indicator of the smooth muscle cells (SMCs) differentiation (Osborn & Weber, 1983; Skalli *et al*., 1986). It has been successfully used as a marker of differentiation of the SMCs surrounding the prenatal bovine extra testicular excurrent system of ducts (Alkafafy & Sinowatz, 2012).

Although there are many studies on the histochemistry of the reproductive tracts, especially epididymal duct in dromedary bulls (Ibrahim *et al*., 2017; Alkafafy, 2021, 2022), a little attention has been paid for ED (Ahmed *et al*., 2013; Ibrahim, 2015). Thus, the main aim of the current research is to apply immunohistochemistry to assess the impact of the sexual seasonality and maturity on the structural and functional properties of the ED; via analyzing the expression levels of S100 and a-SMA proteins within the ED during the periods of sexual activity and inactivity, and during the transitional periods as well.

MATERIAL AND METHOD

Animals and tissues. For each season, specimens of efferent ductular tissues were taken from 7 adult (average age of 9 years) and 7 juvenile (average age 2 years), clinically healthy, male one-humped camels (*Camelus dromedarius*) slaughtered at the local abattoir in Cairo, Egypt. Specimens were taken immediately after slaughter.

Histological techniques. Specimens of ductular tissue were subjected to fixation in neutral buffered formalin, dehydration in ascending grades of ethanol, clearing in xylene, embedding in paraffin wax and sectioning into 5 µm thick sections. Tissue sections were mounted on coated glass slides. Sections were stained with hematoxylin and eosin as described by Alkafafy & Sinowatz (2012).

Immunohistochemical techniques. For immunostaining, sections of ED were dewaxed and rehydrated. The activity of the endogenous peroxidases was stopped by adding 1% hydrogen peroxide (H_2O_2) for 15 min. Then the antigens were retrieved using heat conducted by a microwave oven (700 w). Thus, sections were immersed in 0.01 mol/L citrate buffer (pH 6) and heated for 10 min. Blocking of non-specific bindings in sections by 5 % bovine serum albumin (BSA) in phosphate buffered saline (PBS) for an hour. Incubation of sections in humidified chamber with the specific primary antibodies (Table I) for 1 h at room temperature. The sections were washed by PBS and incubated with the specific secondary antibodies (Table I) for 30 min at room temperature. The sections were washed by PBS for 10 min. Then the secondary antibodies was detected with Vectastain ABC kit (Vector Laboratories Inc., USA), then washed by PBS and the color was developed using DAB reagent (Sigma-Aldrich, St. Louis, MO, USA). Nuclei was stained by immersing the sections in hematoxylin as a counterstain, for 30 s (Alkafafy & Sinowatz, 2012).

Positive and negative controls. Negative controls were applied by omitting the primary or secondary antisera or the ABC reagent and confirmed that no positive staining. Positive controls were conducted following the manufacturers' instructions.

Scoring and photomicrography. The immunoreactivity was assessed via a semi-quantitative individual scoring by three independent observers, blind to the experimental design. Photomicrographs were taken by an imaging system, assembled from of a light microscope (Leica DM LB, Wetzlar, Germany) and a digital camera (Leica EC3, Heerbrugg, Switzerland).

RESULTS

Immunohistochemical findings

S100 immunoreactivity (Table II). During the breeding season (winter), the sections of ED from adult dromedaries showed ciliated cells with a strong S100 IR (Fig. 1a). During non-breeding season (summer) the S100 IR was reduced to weak or even negative (Fig. 1c). Throughout the transitional periods from activity to inactivity (spring) or from inactivity to activity (autumn), ciliated cells exhibited a weak to moderate IR (Figs. 1b,d, respectively). On the other hand,

Table I. Primary and secondary antibodies: identity, sources, and working dilutions.

Primary antibodies					Secondary antibodies		
Against	Origin	Source	Dilution	IncubationTime	Type	Source	Dilution
S100	Rabbit	Dako, Hamburg	1:400	1/2 h at room temperature	Biotinylated pig anti-rabbit IgG	Dako, Hamburg	1:300
α-SMA	Mouse	Dako, Hamburg	1:200	1 h at room temperature	Biotinylated rabbit anti-mouse IgG	Dako, Hamburg	1:300

Table II. Effect of seasonality and sexual maturity on S100-IR in the camel ED.

Season	Sexual maturity	Epithelium		Interstitium	
		CC	NC	PMC	Bl. Vs
Winter	Juvenile	+++	–	±	–
	Adult	+++	–	±	–
Spring	Juvenile	++	–	±	–
	Adult	+/++	–	±	–
Summer	Juvenile	+/++	–	–	–
	Adult	±	–	–	–
Autumn	Juvenile	++	–	±	–
	Adult	+/++	–	±	–

Ciliated cell (CC); Non-ciliated cell (NC); Peritubular muscle coat (PMC); Blood vessels (Bl. Vs). Negative (–); negative to weak (±); weak (+); weak to moderate (+/++); moderate (++) and strong (+++) reaction.

ciliated cells in ED sections from juvenile male camels displayed a nearly consistent S100 IR, reduced from strong (in winter) to moderate (in summer) (Fig. 2).

Meanwhile, the non-ciliated cells within the same sections from both adult and juvenile male camels displayed a negative reactivity throughout the year (Figs. 1 and 2). The SMCs surrounding the ductal epithelium showed a negative to weak S100-IR (in winter), which is gradually reduced and totally disappeared during the non-breeding season (in summer) season. The blood vessels within the interstitium of the ED failed to develop S100-IR throughout the year (Figs. 1 and 2).

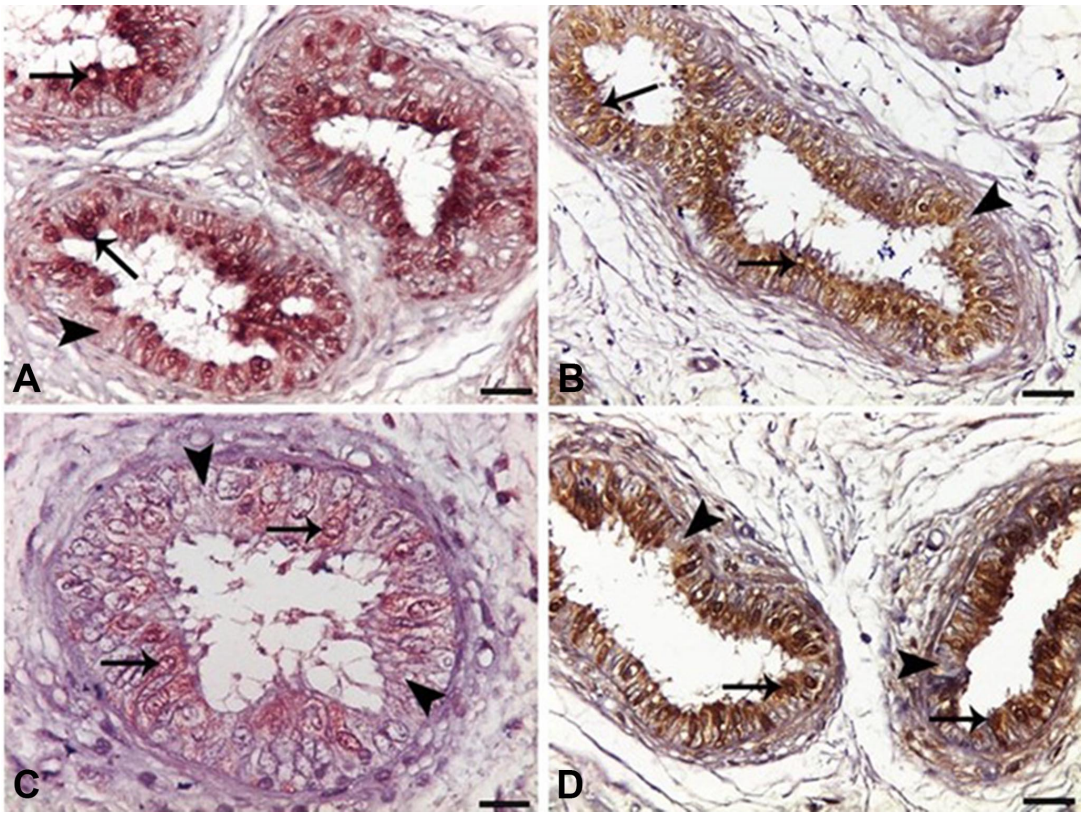

Fig. 1. S100-immunostained ED sections from adult camels during a, winter showing strongly reactive ciliated cells and negative non-ciliated cells; b, spring showing moderately reactive ciliated cells and negative non-ciliated cells (arrows); c, summer displaying weakly reactive ciliated cells and negative non-ciliated cells; d, autumn displaying moderately reactive ciliated cells and negative non-ciliated cells. Ciliated cells (arrows) and non-ciliated cells (arrowheads). Scale bars: 50 μm (a, b, and d) and 25 μm (c).

Alpha-smooth muscle actin immunoactivity (Table III). The SMCs building the periductular muscular coat (PMC) in sections from the ED from adult male camels displayed a variable immunoreactivity against the a-SMA throughout the year. This reactivity was maximum (Fig. 3a) during breeding season (in winter) and minimum or weak (Fig. 3b) during non-breeding season (in summer). Yet, the reactivity against α-SMA was moderate during the transition periods (in spring and autumn) intermittent between the sexual activity and inactivity (Figs. 3c,d). On the other hand, the periductal SMCs in sections from juvenile ED displayed a moderate reactivity against a-SMA throughout the year (Fig. 4). The interstitial blood vessels showed a variable reactivity ranging from strong (in winter), moderate (in spring and autumn) to weak (in summer) in sections of adult ED (Figs. 3a-d). Similar findings were also reported in sections from juvenile ED (Figs. 4a-d).

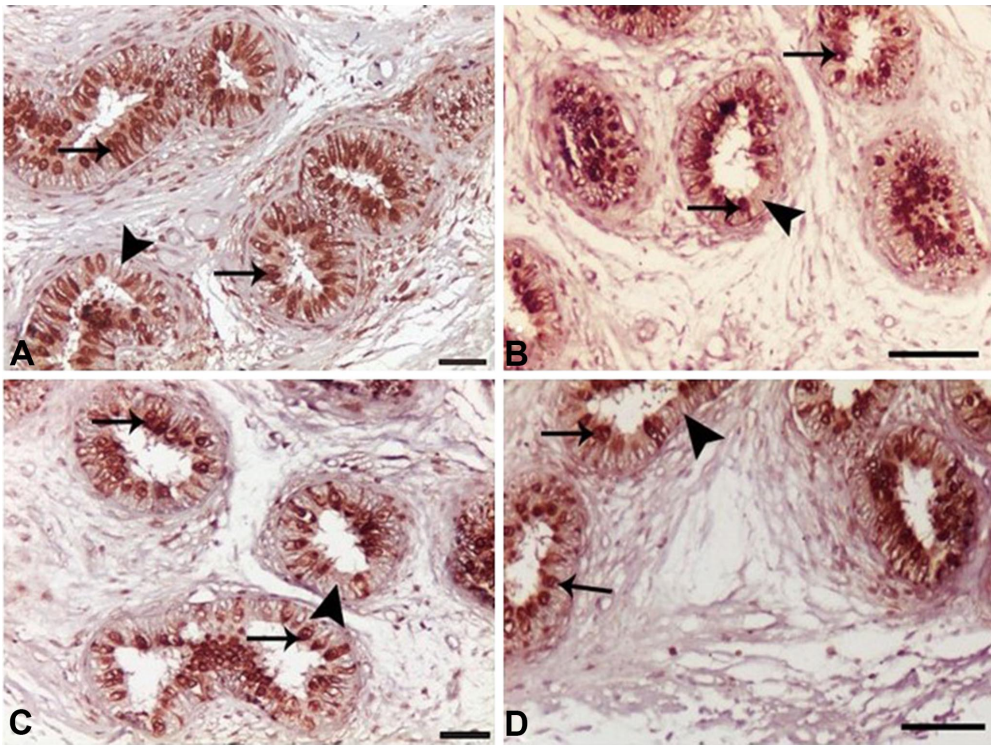

Fig. 2. S100-immunostained ED sections from juvenile camels during a, winter; b, spring; c, summer and d, autumn, showing moderate to strongly reactive ciliated cells (arrows) and negative non-ciliated cells (arrowheads). Scale bars: 50 µm (a & c) and 100 µm (b & d).

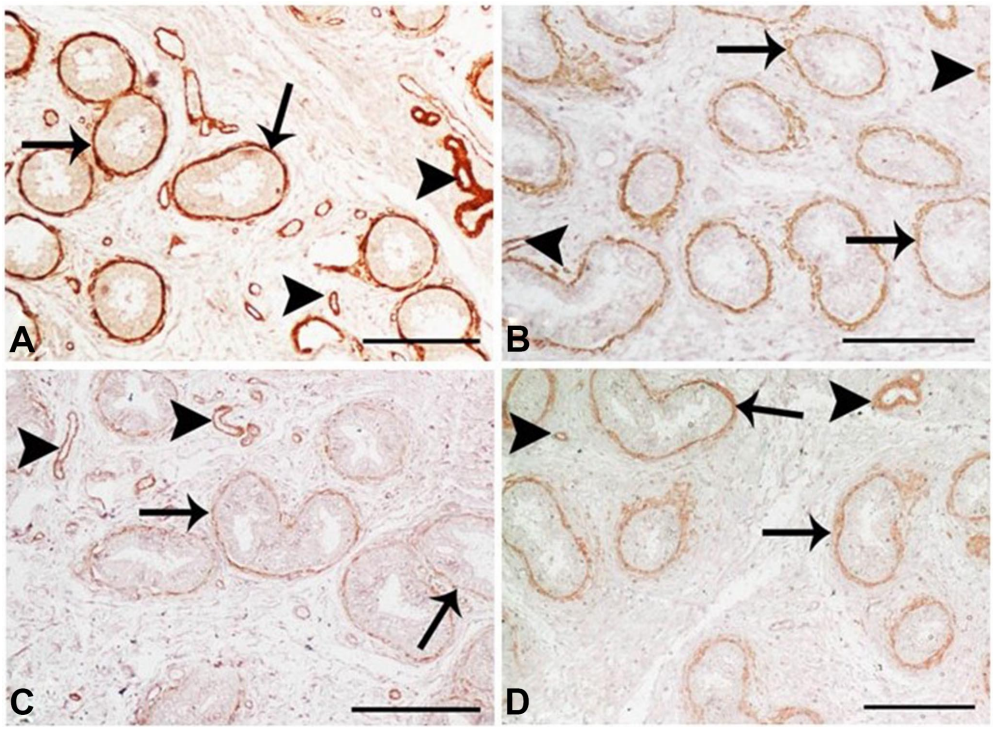

Fig. 3. Alpha-SMA-immunostained ED sections from adult camels during a, winter showing strongly reactive peritubular (arrows) and vascular (arrowheads) smooth muscle cells (SMCs); b, summer displaying weakly reactive peritubular (arrows) and vascular (arrowheads) SMCs c, spring and d, autumn showing moderately reactive peritubular (arrows) and vascular (arrowheads) SMCs. Scale bars: 100 µm.

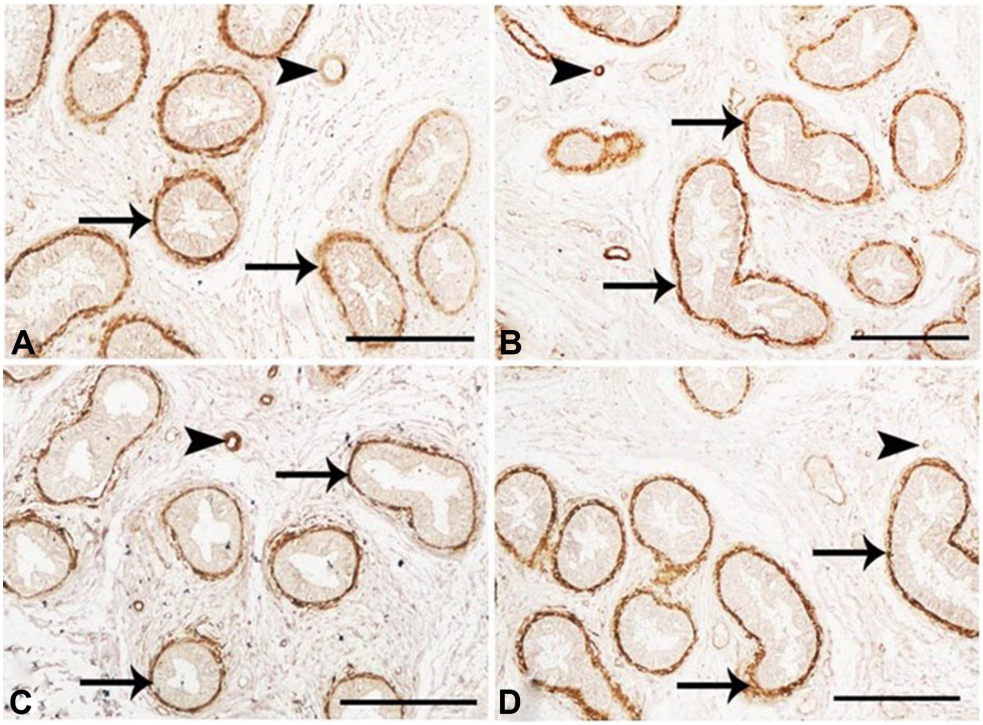

Fig. 4. Alpha-SMA-immunostained ED sections from juvenile camels during a, winter; b, spring; c, summer and d, autumn, showing moderately reactive peritubular (arrows) and vascular (arrowheads) SMCs throughout the year. Scale bars: 100 μm.

Table III. Effect of seasonality and sexual maturity on α-SMA-IR in the camel ED.

Season	Sexual maturity	Epithelium		Interstitium	
		CC	NC	PMC	Bl.Vs
Winter	Juvenile	–	–	++	++
	Adult	–	–	+++	+++
Spring	Juvenile	–	–	++	++
	Adult	–	–	++	++
Summer	Juvenile	–	–	++	++
	Adult	–	–	+	+
Autumn	Juvenile	–	–	++	++
	Adult	–	–	++	++

Ciliated cell (CC); Non-ciliated cell (NC); Peritubular muscle coat (PMC); Blood vessels (Bl. Vs). Negative (–); weak (+); weak to moderate (+/++); moderate (++) and strong (+++) reaction.

DISCUSSION

The sections of the ED from adult camels in breeding season displayed an alternative pattern of S100-IR, between the distinctly positive ciliated and the negative non-ciliated cells. These findings agree with a previous report in camels (Ibrahim, 2015). The pattern of S100-IR in the camel ED was maintained but with a weaker signal (periods of transition) or even non-reactivity (periods of sexual inactivity). This may be due to seasonal and cyclic androgen-dependent alterations (Ibrahim *et al*., 2017). Proteins of S100 are involved in many activities of the cell. These comprise regulation of transmembrane movement of the univalent cations and the modulation of the cell membranes' physical state. The bioactivities of the S100 proteins in extra testicular excurrent duct system are still not fully understood, yet they may impact both the secretory and the absorptive functions. These were reported by Cruzana *et al*. (2003) in the intratesticular excurrent duct system. Additionally, it may be assumed that S100 may contribute to the events of cell contractility and transcytotic activities (Czykier *et al*., 2000).

Though the endothelial cells and the vascular SMCs of the blood vessels within the interstitium of the ED failed to develop S100-IR throughout the year, the SMCs surrounding the ductal epithelium showed a variable reactivity ranged from weak S100-IR (in breeding season) to totally absent in the non-breeding season. These findings go in line with our previous research on camel epididymis during breeding season (Alkafafy *et al*., 2011), but not with the findings reported in European bison by Czykier *et al*. (2010), who assumed that this reactivity is not correlated with age or maturity. This discrepancy may be due to species-specific variations (Abd-Elmaksoud *et al*., 2014). S100 proteins accomplish many critical roles to regulate Ca^{2+} homeostasis and are substantial molecules to transduce Ca^{2+} signaling via interaction with diverse types of ligand proteins

in SMCs promoting their contractility (Czykier *et al*., 2000; Heizmann *et al*., 2002). The transductal transport of sperms is basically dependent on the contraction of the peritubular SMCs (Hinton, 2010).

The ciliated cells in ED sections from juvenile male camels showed more or less a steady reactivity ranged from strong (in winter) to moderate (in summer). However, the non-ciliated cells within the same sections from juvenile male camels displayed a negative to weak reactivity throughout the year. Additionally, it has been reported that the epithelial cells of the prenatal bovine ED subject to cytodifferentiation as early as the 3rd gestational month, and proceeding distalward (Wrobel, 2001; Alkafafy & Sinowatz, 2012). Accordingly, the columnar epithelium lining the proximal portions of the excurrent duct system, especially the segment corresponding to the ED are actually distinguished into ciliated (propulsive) and nonciliated (absorptive and/or secretory) cells (Alkafafy & Sinowatz, 2012). These modifications concur with the prenatal growth of the Leydig cells and the associated elevation in the fetal androgen levels (Rüsse & Sinowatz, 1991). Analogous to the findings reported in the bovines, previous records were also reported in rhesus monkeys (Alexander, 1972) and in humans (Zondek & Zondek, 1980). Likewise, the ED in juvenile camels may subject to androgen-dependent postnatal modifications. Moreover, the current findings in ED from juvenile male camels may be attributed to consistent and lower androgen levels throughout the year.

The SMCs forming the PMC of the ED displayed a variable α-SMA immunoreactivity in adult male camels throughout the year. This reactivity reached the peak in the breeding season and gradually faded out during periods of transition, reaching its lowest magnitude during the non-breeding season. The current findings are in parallel to that reported by Ibrahim (2015) in camel ED and Ibrahim *et al*. (2017), in camel epididymis. Additionally, the periductal SMCs in sections from juvenile ED showed a moderate reactivity against a-SMA throughout the year. It is worth noting that α-SMA is an isoform of actin, which belongs to a class of proteins possessing contractility. It is a reliable marker for differentiation of the SMCs (Osborn & Weber, 1983; Skalli *et al*., 1986). It has been used as a marker of differentiation of the SMCs surrounding the prenatal bovine extra testicular excurrent system of ducts (Alkafafy & Sinowatz, 2012). Additionally, it is expressed by the SMCs surrounding the ED earlier than those of the epididymal duct of the same fetus. The SMCs surrounding both the prenatal ED and epididymal duct displayed a concentric pattern, where the highly differentiated cells are closer to the basement membranes of the tubules (Alkafafy & Sinowatz, 2012). In this regard, Francavilla *et al*. (1987), mentioned that the peritubular cells undergo a process of cytodifferentiation coinciding with the advanced increase of α-SMA-IR (Francavilla *et al*., 1987). The immunoreactivity for α-SMA possesses both spatial and temporal correlation with the existence of contractile filaments. The appearance of the contractile elements begins within the peritubular cells closer to the epithelium and gradually spread outwards (Francavilla *et al*., 1987). Our current findings in the ED from juvenile camels go in line with this trend. Additionally, Schlatt *et al*. (1993), reported that the differentiation of the SMCs surrounding the tubules in the testis of monkey during the process of the sexual maturity is dependent on the androgen and is retained in adults even after the hormonal withdrawal.

Also, the interstitial blood vessels showed a variable reactivity ranged from strong in winter (breeding season) to weak in summer (non-breeding season) in sections of ED from both adults and juveniles. These findings agree with those reported by Skalli *et al*. (1989), who stated that α-SMA is an isoform specific to SMCs and exists in high quantities in the SMCs of the blood vessels.

Furthermore, the current immunohistochemical findings showed seasonal variations in the S100-IR and α-SMA-IR in adult camel ED. These variations agree with previous studies on camels (Ibrahim, 2015; Ibrahim *et al*., 2017) and on prenatal bovines (Alkafafy & Sinowatz, 2012). Also, our current findings go in line with the cyclic expression of S100 and α-SMA proteins in the poll glands in adult male camels (Ibrahim *et al*., 2020) and in the mammary gland of she-camels during lactation (strong IR) and non-lactation (weak IR) periods (Helal *et al*., 2013). Regarding the tissues which are dependent on the levels of androgens and are located distant from the male reproductive organs, it is worth noting that the poll gland is typical gland that exhibit a seasonal activity concur with the rut season (Ebada *et al*., 2012). Accordingly, the poll glands displayed a strong immunoreaction with antibodies against both of S-100 and α-SMA during breeding season when compared to those during non-breeding season (Ibrahim *et al*., 2020). Taken together, a similar cyclic pattern of expression of S100 and α-SMA proteins could be seen in the dromedary ED during breeding and non-breeding seasons. Consequently, the expression of both S100 and α-SMA proteins in the ED may be dependent on androgens, controlling the cyclic changes in adults and the differentiation processes in juveniles.

In conclusion, the seasonal variation in the immunoexpression of both S100 and α-SMA in the male camels ED is proportional to the sexual maturity and increases during the periods of reproductive activity. This

positive correlation between the sexual maturity and breeding seasonality from one side and the level of immunoreactivity from the other side, is mostly regulated by androgens. This suggests a crucial role of both a-SMA and S100 proteins in the regulation of the diverse functions of the ED in male dromedaries

ACKNOWLEDGEMENT. The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-109).

ALKAFAFY, M. E. Impacto de la madurez sexual y la estacionalidad en la inmunolocalización de S100 y actina de músculo liso alfa en los conductillos eferentes del camello dromedario. *Int. J. Morphol., 42(5)*:1347-1354, 2024.

RESUMEN: El estudio fue diseñado para explorar la asociación entre la inmunolocalización de la actina de músculo liso alfa (α-SMA) y las proteínas S100 dentro de los conductos eferentes (ED) por una pate, y la madurez sexual y estacionalidad por otra parte. Para cada estación, se obtuvieron muestras de tejido de 7 dromedarios machos adultos jóvenes y clínicamente sanos. Las muestras se investigaron mediante procedimientos inmunohistoquímicos estándar. Los hallazgos inmunohistoquímicos mostraron que el patrón de expresión era similar para ambas proteínas, ya sea en adultos o en jóvenes. La expresión fue significativamente mayor en adultos durante la temporada de actividad sexual (invierno), bastante reducida durante los períodos de transición de actividad a inactividad (primavera) o de inactividad a actividad (otoño) y alcanzaba su magnitud más baja durante la temporada de inactividad sexual (verano). Por otro lado, una inmunorreactividad moderada de ambas proteínas en el DE de camellos machos juveniles fue casi uniforme durante todo el año. La inmunorreactividad tanto para α-SMA (células musculares lisas en las paredes ductulares y vasculares) como para S100 (células ciliadas ductulares), fue evidenciada durante los períodos de actividad sexual. Se puede concluir que la inmunorreactividad es dependiente de los andrógenos y se correlaciona positivamente tanto con la actividad sexual como con la madurez, lo que sugiere un papel crucial de las proteínas α-SMA y S100 en la regulación de las diversas funciones del DE en dromedarios machos.

PALABRAS CLAVE: Camello dromedario; Conductos eferentes, α-SMA; S100; Inmunohistoquímica.

REFERENCES

Abd-Elmaksoud, A. S.; Shoeib, M. B. & Marei, H. E. S. Localization of S-100 proteins in the testis and epididymis of poultry and rabbits. *Anat. Cell Biol., 47(3)*:180-7, 2014.

Abdel-Maksoud, F. M.; Hussein, M. T. & Attaai, A. Seasonal variation of the intraepithelial gland in camel epididymis with special reference to autophagosome. *Microsc. Microanal., 25(4)*:1052-60, 2019.

Ahmed, Y.; El-Sakhawy, M.; El-Shammaa, M.; El-Sabaa, A.; Hussein, S. & Alkafafy, M. Histological and histochemical studies of the efferent ductules of male one humped camel (*Camelus dromedarius*). *J. Am. Sci., 9(3)*:48-55, 2013.

Alexander, N. J. Prenatal development of the ductus epididymidis in the rhesus monkey. The effects of fetal castration. *Am. J. Anat.,135(1)*:119-33, 1972.

Alkafafy, M. & Sinowatz, F. Prenatal development of the bovine epididymis: light microscopical, glycohistochemical and immunohistochemical studies. *Acta Histochem., 114 (7)*:682-94, 2012.

Alkafafy, M. E. Application of lectins for detection of glycomic differences in the epididymal duct in water buffalo (*Bubalus bubalis*) and dromedary camel (*Camelus dromedarius*). *Int. J. Morphol., 40(3)*:662-71, 2022.

Alkafafy, M. E. Immunoreactivity of alpha smooth muscle actin in the epididymis of the dromedary camel: impact of the sexual maturity and the breeding seasonality. *J. Camel Pract. Res., 28(1)*:33-7, 2021.

Alkafafy, M.; Rashed, R.; Emara, S.; Nada, M. & Helal, A. Histological and immunohistochemical studies on the epididymal duct in the dromedary camel (*Camelus dromedarius*). *Anat. Cell Biol., 44(4)*:284-94, 2011.

Cruzana, M.B.; Budipitojo, T.; De Ocampo, G.; Sasaki, M.; Kitamura, N. & Yamada, J. Immunohistochemical distribution of S-100 protein and subunits (S100-alpha and S100-beta) in the swamp-type water buffalo (*Bubalus bubalis*) testis. Andrologia, 35(3):142-5, 2003.

Czykier, E.; Sawicki, B. & Zabel, M. S-100 protein immunoreactivity in mammalian testis and epididymis. *Folia Histochem. Cytobiol., 38(4)*:163-6, 2000.

Czykier, E.; Zabel, M.; Surdyk-Zasada, J.; Lebelt, A. & Klim, B. Assessment of S100 protein expression in the epididymis of juvenile and adult European bison. *Folia Histochem. Cytobiol., 48(3)*:333-8, 2010.

Ebada, S.; Helal, A. & Alkafafy, M. Immunohistochemical studies on the poll gland of the dromedary camel (*Camelus dromedarius*) during the rutting season. *Acta Histochem., 114(4)*:363-9, 2012.

Farah, Z. *An Introduction to the Camel. In: Farah, Z. & Fischer, A. (Eds.). Milk and Meat from the Camel-Handbook on Products and Processing.* Zürich, Hochschulverlag, 2004. pp.15-28.

Francavilla, S.; Moscardelli, S.; Properzi, G.; De Matteis, M. A.; Scorza Barcellona, P.; Natali, P.G. & De Martino C. Postnatal development of epididymis and ductus deferens in the rat. A correlation between the ultrastructure of the epithelium and tubule wall, and the fluorescence-microscopic distribution of actin, myosin, fibronectin, and basement membrane. *Cell Tissue Res., 249(2)*:257-65, 1987.

Heizmann, C. W.; Fritz, G. & Schäfer, B. W. S100 proteins: structure, functions, and pathology. *Front. Biosci., 7*:d1356-68, 2002.

Helal, A.; Emara, S. & Alkafafy, M. Immunohistochemical studies on the dromedary camel (Camelus dromedaries) mammary gland during lactation and non-lactation periods. *J. Camel Pract. Res., 20(2)*:175-82, 2013.

Hinton, B. T. *What Does the Epididymis Do and How Does It Do It?* In: Robaire, B. & Chan, P. (Eds.). Handbook of Andrology. Lawrence (KS), The American Society of Andrology, Allen Press, 2010.

Ibrahim, D. & Abdel-Maksoud, F. Immunohistochemical and ultrastructural features of the seasonal changes in the epididymal epithelium of Camel (*Camelus dromedarius*). *Microsc. Microanal., 25(5)*:1273-82, 2019.

Ibrahim, Z. H. Morphometric and immunohistochemical studies on camel efferent ductules in relation to reproductive activity. *Nova J. Med. Biol. Sci., 4(4)*:1-7, 2015.

Ibrahim, Z. H.; Al-Kheraije, K. A. & El-Tigani-Asil, E. A. Seasonal studies on morphology and immunohistochemical localisation of s-100 and alpha smooth muscle actin proteins in poll glands of dromedary camel. *J. Camel Pract. Res., 27(1)*:39-47, 2020.

Ibrahim, Z. H.; Al-Kheraije, K. A. & Singh, S. K. Morphological and histochemical changes in the dromedary camel epididymis in relation to reproductive activity. *Histol. Histopathol., 36(5)*:485-504, 2021.

Ibrahim, Z. H.; Joshi, D. & Singh, S. K. Seasonal immunohistochemical reactivity of S-100 and a-smooth muscle actin proteins in the epididymis of dromedary camel, *Camelus dromedarius*. *Andrologia, 49(6)*:e12667, 2017.

Ibrahim, Z. S.; Alkafafy, M.; Soliman, M. M. & Ahmed, M. M. Molecular mechanism of hepato-renal protection of camel milk against oxidative stress-perturbations. *J. Camel Pract. Res., 23(1)*:53-63, 2016.

Ilio, K. Y. & Hess, R. A. Structure and function of the ductuli efferentes: a review. *Microsc. Res. Tech., 29(6)*:432-67, 1994.

Osborn, M. & Weber, K. Tumour diagnosis by intermediate filament typing: a novel tool for surgical pathology. *Lab. Invest., 48(4)*:372-94, 1983.

Rüsse, I. & Sinowatz, F. *Lehrbuch der Embryologie der Haustiere.* Berlin, Verlag Paul Parey, 1991. pp.304-37.

Schlatt, S.; Weinbauer, G. F.; Arslan, M. & Nieschlag, E. Appearance of alpha-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. *J. Androl., 14(5)*:340-50, 1993.

Singh, U. B. & Bharadwaj, M. B. Histological studies on the testicular seminal pathway and changes in the epididymis of the camel (*Camelus dromedarius*). Part IV. *Acta Anat. (Basel), 108(4)*:481-9, 1980.

Skalli, O.; Pelte, M. F.; Peclet, M. C.; Gabbiani, G.; Gugliotta, P.; Bussolati, G.; Ravazzola, M. & Orci L. Alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, is present in microfilamentous bundles of pericytes. *J. Histochem. Cytochem., 37(3)*:315-21, 1989.

Skalli, O.; Ropraz, P.; Trzeciak, A.; Benzonana, G.; Gillessen, D. & Gabbiani, G. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. *J. Cell Biol., 103(6)*:2787-96, 1986.

Tingari, M. D. & Moniem, K. A. On the regional histology and histochemistry of the epididymis of the camel (*Camelus dromedarius*). *J. Reprod. Fertil., 57(1)*:11-20, 1979.

Tingari, M. D. The fine structure of the epithelial lining of the epididymis of the camel (*Camelus dromedarius*) with special reference to regional differences. *J. Anat., 165*:210-4, 1989.

Tingari, M. D.; Ramos, A. S.; Gaili, E. S. E.; Rahma, B. A. & Saad, A. H. Morphology of the testis of the one humped camel in relation to reproductive activity. *J. Anat., 139(Pt. 1)*:133-43, 1984.

Zayed, A. E.; Aly, K.; Ibrahim, I. A. & Abd El-Maksoud, F. M. Morphological studies on the seasonal changes in the epididymal duct of the one-humped camel (*Camelus dromedarius*). *Vet. Sci. Dev., 2(1)*:e3, 2012.

Zayed, A. E.; Hifny, A.; Abou-Elmagd, A. & Wrobel, K. H. Seasonal changes in the intertubular tissue of the camel testis (*Camelus dromedarius*). *Ann. Anat., 177(3)*:199-212, 1995.

Zimmer, D. B.; Eubanks, J. O.; Ramakrishnan, D. & Criscitiello, M. F. Evolution of the S100 family of calcium sensor proteins. *Cell Calcium, 53(3)*:170-9, 2013.

Zondek, L. H. & Zondek, T. Normal and abnormal development of the epididymis of the foetus and infant. *Eur. J. Pediatr., 134(1)*:39-44, 1980.

Corresponding author:
Mohamed E. Alkafafy
Department of Biotechnology
College of Science
Taif University
P.O. Box 11099
Taif 21944
SAUDI ARABIA

E-mail: m.kafafy@tu.edu.sa